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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/019980 A1

(54) Title: VACCINE AGAINST YERSINIA COMPRISING ONE OR TWO ANTIBODIES, ONE SPECIFIC FOR *YERSINIA PESTIS* F1-ANTIGEN AND THE OTHER ONE FOR *YERSINA PESTIS* V-ANTIGEN

(57) Abstract: The use of (i) an antibody specific for *Yersinia pestis* F1-antigen, or a binding fragment thereof, or (ii) an antibody specific for *Yersinia pestis* V-antigen, or a binding fragment thereof, or a combination of (i) and (ii), in the production of a medicament for the treatment of infection by *Yersinia pestis*. It has been found that such treatments are effective therapies for *Yersinia pestis* infection. In addition, the combination produces a synergistic effect when used prophylactically.

VACCINE AGAINST YERSINIA COMPRISING ONE OR TWO ANTIBODIES, ONE SPECIFIC FOR YERSINIA PESTIS F1-ANTIGEN AND THE OTHER ONE FOR YERSINIA PESTIS V-ANTIGEN

The present invention relates to antibodies, which are administered to a human or animal prophylactically or as a therapy.

In particular, the present invention relates to antibodies, which act synergistically when administered prophylactically and when administered as a therapy.

The present invention also relates to novel vaccines and the protection and treatment against the organism *Yersinia pestis*. Such vaccines are capable of offering protection against bubonic and pneumonic plague.

Yersinia pestis, the causative agent of plague, has accounted for the deaths of millions of people throughout recorded history. The second pandemic (The Black Death) is thought to have killed an estimated 17 million to 28 million Europeans between the 14th and 17th centuries. The third pandemic, believed to have started in the Yunan Province of China in the 1850s, has lead to the worldwide spread of plague, which is now endemic to several regions including Africa, India and the South Western states of the USA (Perry, R. D. et al. 1997. Clinical Microbiology Reviews **10**:35-66). Despite the current low incidence of plague, the bacterium resides in natural animal reservoirs and regular, though relatively small outbreaks of plague occur (Duplantier, J. M. et al. 2001. Bulletin De La Societe De Pathologie Exotique **94**:119-122; Migliani, R. et al. 2001.Bulletin De La Societe De Pathologie Exotique **94**:115-118; Ratsitorahina, M. et al. 2000. Lancet **355**:111-113).

Improvements in transport links between endemic areas and large population centres bring with it the potential for large-scale plague outbreaks, highlighted by the recent outbreak in India (Shivaji, S. et al. 2000. Fems Microbiology Letters **189**:247-

Next-generation plague sub-unit vaccines are being developed, based on the recombinant F1-antigen (F1) and low calcium response V-antigen (LcrV) proteins, derived from *Y. pestis*. Immunisation with either protein provides protection against pneumonic or bubonic disease in animal models of infection (Heath, D. G. et al. 1998. Vaccine **16**:1131-1137; Leary, S. E. C. et al. 1995. Infection and Immunity **63**:2854-2858; Williamson, E. D. 2001. Journal of Applied Microbiology **91**:606-608) but greater than additive protection is achieved when F1 and LcrV are combined, with protection against up to 10^5 median lethal doses (MLD) of *Y. pestis* reported (Williamson, E. D. et al. 1995. Fems Immunology and Medical Microbiology **12**:223-230). Such vaccines must be administered prior to exposure, and multiple doses are required. Although strategies to reduce the time to immunity and the number of vaccine doses have shown promise (Williamson, E. D. et al. 2000. A single dose sub-unit vaccine protects against pneumonic plague. Vaccine **19**:566-571), it is unlikely that vaccination will provide post-exposure protection against disease.

There is therefore a need for fast-acting anti-plague treatments to provide rapid therapy, particularly in the event that drug resistant strains of *Y. pestis* are involved.

Previously, F1-04-A-G1, a mouse monoclonal antibody raised against F1 was shown to protect mice in models for bubonic and pneumonic plague (Anderson, G. W. et al. 1997. American Journal of Tropical Medicine and Hygiene **56**:471-473). Also, preliminary studies showed that an LcrV-specific monoclonal antibody (Mab 7.3) protected mice in a bubonic plague model (Hill, J. et al. 1997. Infection and Immunity **65**:4476-4482).

Although antisera have been used to treat a range of diseases caused by other pathogens (Keller M. A. et al. 2000. Clin. Microbiol. Rev. 2000 **13**:602-14), neither antisera nor monoclonal antibodies have been previously proposed as a treatment for

to region 168-275 (Motin, V. L. et al. 1994. Infection and Immunity **62**:4192-4201). Similarly, Mab 7.3 used in the present application has been mapped to bind to a conformational epitope between aa 135-275 of LcrV (Hill, J. et al. 1997. Infection and Immunity **65**:4476-4482). Therefore, this central region of LcrV appears to be a good target for antibodies useful in the present invention.

Suitably the medicament is for administration up to about 48 hours post-infection, although longer periods may be envisaged if the dosage is increased sufficiently.

A number of strategies can be used to increase the clinical acceptability of the antibodies or binding fragments (Casadevall, A. 1999. Clinical Immunology **93**:5-15). For example, the specificity of animal antibodies can be transferred to a human antibody framework, a process termed "humanisation" (Taylor, G. et al. 1991. Lancet **337**:1411-1412; Winter, G. et al. 1993. Trends in Pharmacological Sciences **14**:139-143) or animal antibodies can be chemically treated to improve their therapeutic properties (Mayers, C. N. et al. 2001. Reviews in Medical Microbiology **12**:29-37). Alternatively, antibodies can be generated from naïve human single chain antibody libraries (de Haard, H. J. et al. 1999. Journal of Biological Chemistry **274**:18218-18230; Knappik, A. et al. 2000. Journal of Molecular Biology **296**:57-86; Nissim, A. et al. 1994. Embo Journal **13**:692-698) or from immunised transgenic animals that express a human antibody repertoire (Neuberger, M. et al. 1997. Nature **386**:25-26).

In a particularly preferred embodiment, the antibodies or binding fragments thereof used are "humanised" by humanisation as described above, or are fully human antibodies as a result of generation from human libraries, or transgenic animals, also as described above.

subcutaneous, intramuscular or intramuscular dosing or as a suppository for rectal dosing.

The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art.

For example, the pharmaceutical compositions may be in the form of a sterile injectable aqueous or oily suspension, which may be formulated according to known procedures using one or more of the appropriate dispersing or wetting agents and suspending agents.

Compositions for administration by inhalation may be in the form of a conventional pressurised aerosol arranged to dispense the active antibody composition either as an aerosol containing finely divided solid or liquid droplets. Conventional aerosol propellants such as volatile fluorinated hydrocarbons or hydrocarbons may be used and the aerosol device is conveniently arranged to dispense a metered quantity of active ingredient.

For further information on Formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board, Pergamon Press 1990).

The amount of active ingredient that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration. Dosage unit forms will generally contain about 1 mg to about 2g of an active ingredient.

The size of the dose for therapeutic or prophylactic purposes of the compositions of the invention will vary according to the nature and severity of the conditions, the age and sex of the animal or patient and the route of administration, according to

pestis, and these vaccines form a further aspect of the invention.

Yet a further aspect comprises a method of immunising against infection by *Yersinia pestis* comprising administering a vaccine as described above.

In a further aspect, the invention provides the use of a combination of an antibody specific for *Yersinia pestis* F1-antigen, or a binding fragment thereof, and an antibody specific for *Yersinia pestis* V-antigen, or a binding fragment thereof, in the production of a medicament for the passive immunisation of a human or animal against infection by *Yersinia pestis*.

Preferred antibody combinations are as described above in relation to the use in therapy.

LcrV has a key role in type III secretion (TTS) by the *Yersinia spp.*, a process that allows the injection of a set of effector proteins directly into the cytosol of eukaryotic target cells upon intimate contact (Cornelis, G. R. 1998. Journal of Bacteriology **180**:5495-5504; Hueck, C. J. 1998. Microbiology and Molecular Biology Reviews **62**:379-433; Rosqvist, R. et al. 1991. Infection and Immunity **59**:4562-4569; Rosqvist, R. et al. 1994. Embo Journal **13**:964-972). The effector proteins (termed Yops) have a range of function that promote the killing of phagocytic host cells. Protective polyclonal antisera inhibited *Yersinia* TTS in HELA cell cytotoxicity experiments and LcrV was detected at the bacterial surface prior to contact with eukaryotic cells by confocal microscopy analysis (Pettersson, J. A. et al. 1999. Molecular Microbiology **32**:961-976). A similar study showed that Mab 7.3, but not other non-protective Mabs, protected J774 macrophage-like cells against *Yersinia*-mediated killing (Weeks, S. et al. 2002. Mibrobial Pathogenesis **32**:227-237). Antiserum raised against the LcrV homologue of *Pseudomonas aeruginosa* (PcrV) protected mice in a lung infection model and antiserum

1460). By using the invention, both the TTS system and the F1 capsule of the organism are targeted, which might explain the high level of protection observed in the following examples. The present invention will now be described only by way of examples in which reference shall be made to the following Figures in which:-

Figure 1 is a graph showing therapeutic Mab 7.3 treatment of mice challenged with *Y. pestis* via the s.c. (A) and aerosol (B) infection routes. Mice received 35 µg of Mab 7.3 in PBS by i.p. injection 4 hours before or up to 72 hours after challenge, as indicated. Deaths were recorded over a 14 day period. Delayed time to death observed in animals treated with Mab 7.3 at 72 hours (A) and 60 h (B) were statistically significant ($P<0.05$) by Student's T-test analysis compared with untreated control groups

Figure 2 is a graph showing that Mab 7.3 and F1-04-AG-1 display synergy when administered post-infection. Mice were challenged s.c. with 91 MLD *Y. pestis* and treated 48 hours after plague challenge with Mab 7.3 (35 µg), F1-04-A-G1 (100 µg) or both antibodies. Deaths were recorded over a 14 day period.

Example 1

Preparation of Antibodies

Mab 7.3 and F1-04-A-G1 were purified by ammonium sulphate precipitation from hybridoma supernatants. An equal volume of saturated ammonium sulphate solution was added slowly to tissue culture supernatants, followed by overnight stirring at 4°C, then centrifugation at 3,000 *g* for 30 min. The pellets were drained and resuspended in PBS (GIBCO, UK) in 0.1 volumes of the original volume, then dialysed against three changes of PBS. Disposable Econopak columns (BioRad, UK) were packed with protein-G-sepharose beads (Sigma, UK) and antibody solution was passed through the column. The beads were washed with PBS, then antibody was eluted with 50 mM glycine (pH 3) and stored in fractions containing 150 µl Tris HCl (pH 9.1) per 3ml of eluate.

TABLE 1. Dose-dependent protection against bubonic plague with purified Mab 7.3.

Mab 7.3 (µg)*	MLD†	Survivors/group	TTD ± SEM§
35	10	5/6	4.0
10.5	10	5/6	6.0
3.5	10	0/6	8.2±1.1
0.7	10	1/6	4.8±0.5
none	10	0/6	4.8±0.3
35	100	3/6	6.3±0.8
10.5	100	3/6	3.8±2.7
3.5	100	1/6	6.4±1.5
0.7	100	0/6	5.2±0.4
none	100	0/6	4.1±0.3

* Mab 7.3 administered i.p. 24 hours before challenge
† *Y. pestis* administered by s.c. injection in 100 µl PBS
§ Student's T-test: $p<0.05$ compared with PBS control groups receiving the equivalent challenge.

Greater survival was noted in groups given 10.5 µg or 35 µg, compared with those that received 3.5 µg and 0.7 µg of Mab 7.3. The degree of protection was less in animals that received 100 MLD than those injected with 10 MLD (50% and 83% survivors respectively). Therefore, protection against plague was directly proportional to the amount of antibody administered and inversely proportional to the challenge dose.

Five mice received 50 µg Mab 7.3 in 100 µl PBS by intraperitonneal (i.p.) injection and serum levels were determined at different times by anti-LcrV-specific ELISA as described previously (Hill, J. et al. 1997. Infection and Immunity 65:4476-4482). The serum half-life of Mab 7.3 was determined as 5.6 days. The serum antibody level 28 days after dosing was calculated as 2 µg, and five immunised animals were challenged with 18 MLD *Y. pestis* on day 28-post antibody

* 35 µg of Mab 7.3 and/or 100 µg of F1-04-A-G administered by i.p. injection in 100 µg PBS, 4 hours prior to challenge.
† Deaths recorded over a 14 day period

This confirmed the prophylactic properties of F1-04-A-G1 in the pneumonic plague model (Anderson, G. W. et al. 1997. American Journal of Tropical Medicine and Hygiene 56:471-473). Mab 7.3 was less effective as a treatment against s.c. *Y. pestis* challenge than aerosol challenge (Fig. 1), therefore the bubonic plague model chosen for further co-administration studies to test for antibody synergy.

First, antibodies were tested as a pre-treatment against challenge with 50 to 10^5 MLD of *Y. pestis* GB (Table 3).

TABLE 3. Enhanced protection with F1-04-A-G1 and Mab 7.3 as a pre-treatment.

Antibody treatment*	*Y. pestis* challenge (MLD) †	Survivors per group
untreated	50	0/6
F1-04-A-G1 + Mab 7.3	10^2	6/6
F1-04-A-G1 + Mab 7.3	10^3	6/6
F1-04-A-G1 + Mab 7.3	10^4	5/6
F1-04-A-G1 + Mab 7.3	10^5	6/6

* mice were immunised i.p. with 35 µg Mab 7.3 and 100 µg F1-04-A-G1 in PBS.
† s.c plague challenge 4 hours after antibody administration

Surprisingly, protection was observed at all challenge doses; breakthrough was expected at challenge doses greater than 100 MLD (see Table 1 and Anderson, G. W. et al. 1997. American

CLAIMS

1. The use of (i) an antibody specific for *Yersinia pestis* F1-antigen, or a binding fragment thereof, or (ii) an antibody specific for *Yersinia pestis* V-antigen, or a binding fragment thereof, or a combination of (i) and (ii), in the production of a medicament for the treatment of infection by *Yersinia pestis*.

2. The use according to claim 1 wherein a combination of (i) and (ii) is used.

3. The use according to claim 2 wherein the said combination comprises an antibody specific for *Yersinia pestis* F1-antigen, and an antibody specific for *Yersinia pestis* V-antigen.

4. The use according to any one of the preceding claims wherein the antibodies are monoclonal antibodies.

5. The use according to any one of the preceding claims wherein the medicament is for administration up to about 48 hours post-infection.

6. The use according to any one of the preceding claims wherein the antibody specific for *Yersinia pestis* V-antigen or binding fragment thereof specifically binds an epitope of the V-antigen found between amino acids 135-275 of the sequence of the V-antigen.

7. The use according to any one of the preceding claims wherein the antibodies or binding fragments thereof, are humanised.

8. A method of treating a human or animal suffering from the effects of infection with *Yersinia pestis*, said method comprising administering to the human or animal, a therapeutically effective amount of (i) an antibody specific for

17. The use of a combination of an antibody specific for *Yersinia pestis* F1-antigen, or a binding fragment thereof, and an antibody specific for *Yersinia pestis* V-antigen, or a binding fragment thereof, in the production of a medicament for the passive immunisation of a human or animal against infection by *Yersinia pestis*.

18. A use, a method or a composition substantially as hereinbefore described with reference to the accompanying figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03747

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/40 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TITBALL R W ET AL: "Vaccination against bubonic and pneumonic plague" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, no. 30, 20 July 2001 (2001-07-20), pages 4175-4184, XP004255134 ISSN: 0264-410X page 4181, right-hand column, line 15 - line 20 page 4182, left-hand column, line 17 - line 24 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2003	29/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal... Application No
PCT/GB 03/03747

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 73347 A (US NAVY ;BURANS JAMES (US); ALDRICH JENNIFER (US)) 7 December 2000 (2000-12-07) page 4, line 5 -page 8, line 24 ---	1-17
P,X	HILL JIM ET AL: "Synergistic protection of mice against plague with monoclonal antibodies specific for the F1 and V antigens of Yersinia pestis." INFECTION AND IMMUNITY. UNITED STATES APR 2003, vol. 71, no. 4, April 2003 (2003-04), pages 2234-2238, XP002264632 ISSN: 0019-9567 figure 2 ---	1-17
T	JONES S M ET AL: "Protective efficacy of a fully recombinant plague vaccine in the guinea pig" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 21, no. 25-26, 8 September 2003 (2003-09-08), pages 3912-3918, XP004446167 ISSN: 0264-410X abstract -----	1-17

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 18

The subject-matter of claim 18 refers to figures that are not present in the application. The claim 18 is therefore lacking any technical feature allowing a search to be performed.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

PATENT COOPERATION TREATY

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CPG/P/202/WOD		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/03747	29.08.2003	31.08.2002
International Patent Classification (IPC) or both national classification and IPC		
A61K39/40		
Applicant		
THE SECRETARY OF STATE FOR DEFENCE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.03.2004	08.09.2004
Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Le Flao, K Telephone No. +31 70 340-1040

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/03747

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-16 as originally filed

Claims, Numbers

1-16 received on 12.08.2004 with letter of 06.08.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☒ the claims, Nos.: 17,18
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 8,9,15

because:

☒ the said international application, or the said claims Nos. 8,9,15 (method of treatment) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,11-15
	No:	Claims	1-6,8-10,16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-7,10-14,16
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the opinion

The applicant requested, in a fax dated the 21/04/2003, a rectification of an obvious error according to Rule 91.1b. Even if the two missing figures were part of the priority document, the addition of these figures is neither considered as the correction of an obvious error according to Rule 91.1b nor as acceptable amendments according to Rule 70.2(c). Indeed although it is obvious that the figures are missing the rectification itself is not obvious in that one would not immediately realize that nothing else could have been intended than these two figures (Rule 91.1 (b) PCT). The two figures are considered to add subject-matter that is not disclosed in the application as filed (Rule 70.2(c) PCT).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 8, 9 and 15 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: TITBALL R ET AL: 'Vaccination against bubonic and pneumonic plague' VACCINE, vol. 19, no. 30, 20 July 2001, pages 4175-4184, XP004255134 ISSN: 0264-410X

D2: HILL JIM ET AL: 'Regions of Yersinia pestis V antigen that contribute to protection against plague identified by passive and active immunization' INFECTION AND IMMUNITY, vol. 65, no. 11, 1997, pages 4476-4482, XP002264631 ISSN: 0019-9567 cited in the application

D3: WEEKS S ET AL: 'Anti-V antigen antibody protects macrophages from Yersinia pestis -induced cell death and promotes phagocytosis.' MICROBIAL PATHOGENESIS. vol. 32, no. 5, May 2002, pages 227-237, XP001174178 ISSN: 0882-4010

D4: JONES S ET AL: 'Protection conferred by a fully recombinant sub-unit vaccine against Yersinia pestis in male and female mice of four inbred strains' VACCINE, vol. 19, no. 2-3, 15 September 2000, pages 358-366, XP004228846 ISSN: 0264-410X

D5: GREEN M ET AL: 'The SCID/Beige mouse as a model to investigate protection against Yersinia pestis' FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, vol. 23, no. 2, February 1999, pages 107-113, XP001176736 ISSN: 0928-8244

D1 discloses the following : "Evidence has accumulated from a number of studies that antibody plays a key role in protection against plague. Circulating antibody specific for the F1 and V antigens would be able to access the bacterium in its predominantly extracellular existence and bind to surface exposed protein. The observation that a neutralising monoclonal antibody raised to the V antigen could alone protect mice againt live organism challenge underlined the critical role of this virulence factor in the pathogenesis of plague infection. That antibody to F1 + V antigens could be protective against injected whole organism challenge was demonstrated by the passive transfer of F1 + V immune serum from immunised parent strain mice into severe combined immunodeficient recipient strain mice" (D1, p.4181, right-hand column, l.5 - l.20).

D2 discloses monoclonal antibodies raised against recombinant V antigen recognizing epitopes mapped to region I & II, the region II being the major protective region. Monoclonal antibody 7.3, recognizing an epitope in region II (ie amino acids 135-275) passively protected mice against challenge with 12 median lethal doses of Y. pestis GB (D2, see the abstract).

D3 discloses that polyclonal anti V-antigen antibodies and monoclonal antibody 7.3 protected a murine macrophage cell line from Y. pestis-induced cell death (D3, see the abstract).

D4 discloses that passive immunisation with sera raised against partially purified and purified V antigen has been shown to protect against a challenge of up to 100 LD50 with Y. pestis (D4, p.566, right-han column,l.12 - p.567, left-hand column, l.26).

D5 discloses passive immunisation with sera containing anti-F1 and anti-V antibodies protected mice against plague infection (p.108, §2.2, p.109, §2.5, fig.3).

NOVELTY

Claim 1, related to the use of an antibody specific for F1 or for V antigen in the production of a medicament for the treatment of infection by Yersinia pestis & to a method of treatment, is therefore not novel over the disclosure of D1, D2, D3, D4 & D5.

Claims 2 & 3, related to the use of a combination of an antibody specific for F1 and an antibody specific for V antigen are not novel over D1 & D5. Claim 4 is not novel over D1 D2 & D3 since monoclonal antibodies are disclosed in these documents.

Claim 5 is not novel over any of the above cited document since the feature "for administration up to about 48 hours post-infection" is not limiting the scope of the claim. As a matter of fact administrating drugs and determining the dosage regimen and time schedule for administration of drugs belong to non-commercial and non-industrial activities and thus cannot be the subject of a patent claim (Art. 34(4)(a)(i) and Rule 67(1)(iv) PCT).

Claim 6 is not novel over D2 (cf above). Claim 10 is not novel over D1 & D5 disclosing that antibody to F1 + V antigens could be protective against injected whole organism challenge. In D5 it is stated that the serum containing F1 & V specific circulating IgG was administrered i.p. (D5, p.109, §2.5) which anticipates claim 10. Indeed the i.p. administration of the serum is implicitely disclosing a pharmaceutically acceptable carrier defined as "suitable carriers include solid or liquid carriers, such as saline" (p.6 l.24).

Claims 8, 9 & 15 related to method of treatment are not novel for the same reasons as those mentioned for claims 1, 2 & 10.

Claim 7 is novel. Claims 11-15 are novel since they relate to a composition comprising a monoclonal antibody specific for the Y. pestis V -antigen, a monoclonal antibody specific for the Y. pestis F1-antigen and a pharmaceutically acceptable carrier.

INVENTIVE STEP

Claim 7 related to humanised antibodies is formally novel but does not involve an inventive step since it is matter of routine to prepare humanised antibodies.

Claim 11 is not considered to involve an inventive step for the following reasons. Claim 11 differs from D6 in that the antibodies are monoclonal. The effect of the difference is that the antibodies are monoclonal. The problem to be solved by the present invention may therefore be regarded as the provision of a pharmaceutical composition comprising monoclonal antibodies. The solution is solving the problem posed but is not considered to involve an inventive step since it is a matter of routine to prepare monoclonal antibodies and there is an incentive to prepare monoclonal antibodies when effective polyclonal antibodies and relevant epitopes are known. Since D1, D2 & D3 are disclosing neutralizing monoclonal antibodies against the V-antigen that protected mice against live organism challenge it is considered that the skilled person would apply the teaching of any of these documents to the disclosure of D6 thereby arriving to the solution without exercising inventive skill.

Claim 12 is also not considered to involve an inventive step over D6 and D2 since D2 disclosed that monoclonal antibody 7.3 recognizing an epitope in region II (ie amino acids 135-275) passively protected mice against challenge with 12 median lethal doses of Y. pestis GB.

The dependent claims 13-15 do not appear to contain any additional features which, in combination with the features of claim 10 or 11, involve an inventive step as the relevant subject matter is either disclosed in the cited prior art of falls within the knowledge and ability of the skilled person.

INDUSTRIAL APPLICABILITY

For the assessment of the present claims 8, 9, 15 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

CLARITY

The term "or a binding fragment thereof" used in claims 1, 6-8, 10, 12, 13 and 16 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

Yersinia pestis F1-antigen, or a binding fragment thereof, or (ii) an antibody specific for *Yersinia pestis* V-antigen, or a binding fragment thereof, or a combination of (i) and (ii).

9. A method according to claim 8 wherein the method comprises administering a combination of (i) and (ii).

10. A pharmaceutical composition comprising an antibody specific for the *Yersinia pestis* V-antigen or a binding fragment thereof, and an antibody specific for the *Yersinia pestis* F1-antigen or a binding fragment thereof.

11. A pharmaceutical composition according to claim 10 which comprises an antibody specific for the *Yersinia pestis* V-antigen or a binding fragment thereof, and an antibody specific for the *Yersinia pestis* F1-antigen or a binding fragment thereof.

12. A pharmaceutical composition according to claim 10 or claim 11 wherein the antibodies are monoclonal antibodies.

13. A pharmaceutical composition according to any one of claims 10 to 12 wherein the antibody specific for *Yersinia pestis* V-antigen or binding fragment thereof specifically binds an epitope of the V-antigen found between amino acids 135-275 of the sequence of the V-antigen.

14. A pharmaceutical composition according to any one of claims 10 to 13 wherein the antibodies or binding fragments thereof, are humanised or are fully human.

15. A prophylactic vaccine for protection of a human or animal against infection by *Yersinia pestis*, said vaccine comprising a composition according to any one of claims 10 to 14.

16. A method of immunising against infection by *Yersinia pestis* comprising administering a vaccine according to claim 15.

17. The use of a combination of an antibody specific for *Yersinia pestis* F1-antigen, or a binding fragment thereof, and an antibody specific for *Yersinia pestis* V-antigen, or a binding fragment thereof, in the production of a medicament for the passive immunisation of a human or animal against infection by *Yersinia pestis*.

18. A use, a method or a composition substantially as hereinbefore described with reference to the accompanying figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03747

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/40 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TITBALL R W ET AL: "Vaccination against bubonic and pneumonic plague" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, no. 30, 20 July 2001 (2001-07-20), pages 4175-4184, XP004255134 ISSN: 0264-410X page 4181, right-hand column, line 15 - line 20 page 4182, left-hand column, line 17 - line 24 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2003	29/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/GB 03/03747

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HILL JIM ET AL: "Regions of Yersinia pestis V antigen that contribute to protection against plague identified by passive and active immunization" INFECTION AND IMMUNITY, vol. 65, no. 11, 1997, pages 4476-4482, XP002264631 ISSN: 0019-9567 cited in the application abstract	1-17
X	WEEKS S ET AL: "Anti-V antigen antibody protects macrophages from Yersinia pestis -induced cell death and promotes phagocytosis." MICROBIAL PATHOGENESIS. ENGLAND MAY 2002, vol. 32, no. 5, May 2002 (2002-05), pages 227-237, XP001174178 ISSN: 0882-4010 abstract	1-17
X	JONES S M ET AL: "Protection conferred by a fully recombinant sub-unit vaccine against Yersinia pestis in male and female mice of four inbred strains" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, no. 2-3, 15 September 2000 (2000-09-15), pages 358-366, XP004228846 ISSN: 0264-410X page 359, left-hand column, line 3 - line 35	1-17
A	WILLIAMSON E D ET AL: "A single dose sub-unit vaccine protects against pneumonic plague" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 19, no. 4-5, 15 October 2000 (2000-10-15), pages 566-571, XP004218129 ISSN: 0264-410X page 566, right-hand column, line 12 -page 567, left-hand column, line 26	1-17
X	GREEN MICHAEL ET AL: "The SCID/Beige mouse as a model to investigate protection against Yersinia pestis" FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, vol. 23, no. 2, February 1999 (1999-02), pages 107-113, XP001176736 ISSN: 0928-8244 page 108, paragraph 2.2 page 109, paragraph 2.5; figure 3	1-17

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INTERNATIONAL SEARCH REPORT

Internat ..... Application No
PCT/GB 03/03747

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 73347 A (US NAVY ;BURANS JAMES (US); ALDRICH JENNIFER (US)) 7 December 2000 (2000-12-07) page 4, line 5 -page 8, line 24 ---	1-17
P,X	HILL JIM ET AL: "Synergistic protection of mice against plague with monoclonal antibodies specific for the F1 and V antigens of Yersinia pestis." INFECTION AND IMMUNITY. UNITED STATES APR 2003, vol. 71, no. 4, April 2003 (2003-04), pages 2234-2238, XP002264632 ISSN: 0019-9567 figure 2 ---	1-17
T	JONES S M ET AL: "Protective efficacy of a fully recombinant plague vaccine in the guinea pig" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 21, no. 25-26, 8 September 2003 (2003-09-08), pages 3912-3918, XP004446167 ISSN: 0264-410X abstract -----	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/03747

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8, 9 and 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 18
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 18

The subject-matter of claim 18 refers to figures that are not present in the application. The claim 18 is therefore lacking any technical feature allowing a search to be performed.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 03/03747

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0073347 A	07-12-2000	AU 5046700 A	18-12-2000
		WO 0073347 A1	07-12-2000